OECD phase 1A work on a non-spawning fish assay - draft results -

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Non-spawning fish assay

- ✓ To detect chemicals that affect androgenic or estrogenic activity in fish exposed during a limited part of their life-cycle, not including reproduction.
- ✓ Three core biological endpoints:
  - Gross morphology
    2nd sex characters and gonado-somatic index (GSI)
  - Plasma or liver vitellogenin (VTG) levels
  - Histopathology of excised gonads.
- ✓ Test species
  - Fathead minnow (*Pimephales promelas*)
  - Medaka (*Oryzias latipes*)
  - Zebrafish (*Danio renio*)

Outline of non-spawning fish assay

Days after initiation of the exposure

0 14 21

Daily observation on mortality and abnormal response

Separate with mesh barrier

Male × 5
Female × 5

Male × 5
Female × 5

Male × 5
Female × 5

Endpoints

- Sexual characters
- GSI
- VTG level
- Gonad histology

OECD phase 1A validation study

- ✓ To obtain initial information on:

  i) the relevance of the endpoints used in terms of their sensitivity and (species) specificity

  ii) the reproducibility of test results.

- ✓ Participating laboratories
  - CERI (Lead laboratory)
  - Bayer CropScience AG. (Germany)
  - METOCEAN ENVIRONMENT INC. (METO) (Japan)
  - National Institute for Environmental Studies (NIES) (Japan)
- ✓ Test substance
  - 17β-Estradiol (E2) (estrogen)
  - 17β-Trenbolone (TB) (androgen)

Draft results of the studies with E2

Test species	Sex	Main endpoint			
		Gross morphology		VTG induction	Gonad histology
		GSI	2nd sex characters		
Fathead minnow	Male	△	△	◎	NA
	Female	×	×	○	
Medaka	Male	×	△	◎	
	Female	×	×	○	
Zebrafish	Male	×	ND	◎	
	Female	×		○	

◎: Cleary detected in all studies, ○: Detected in all studies, △: Detected in a few studies,

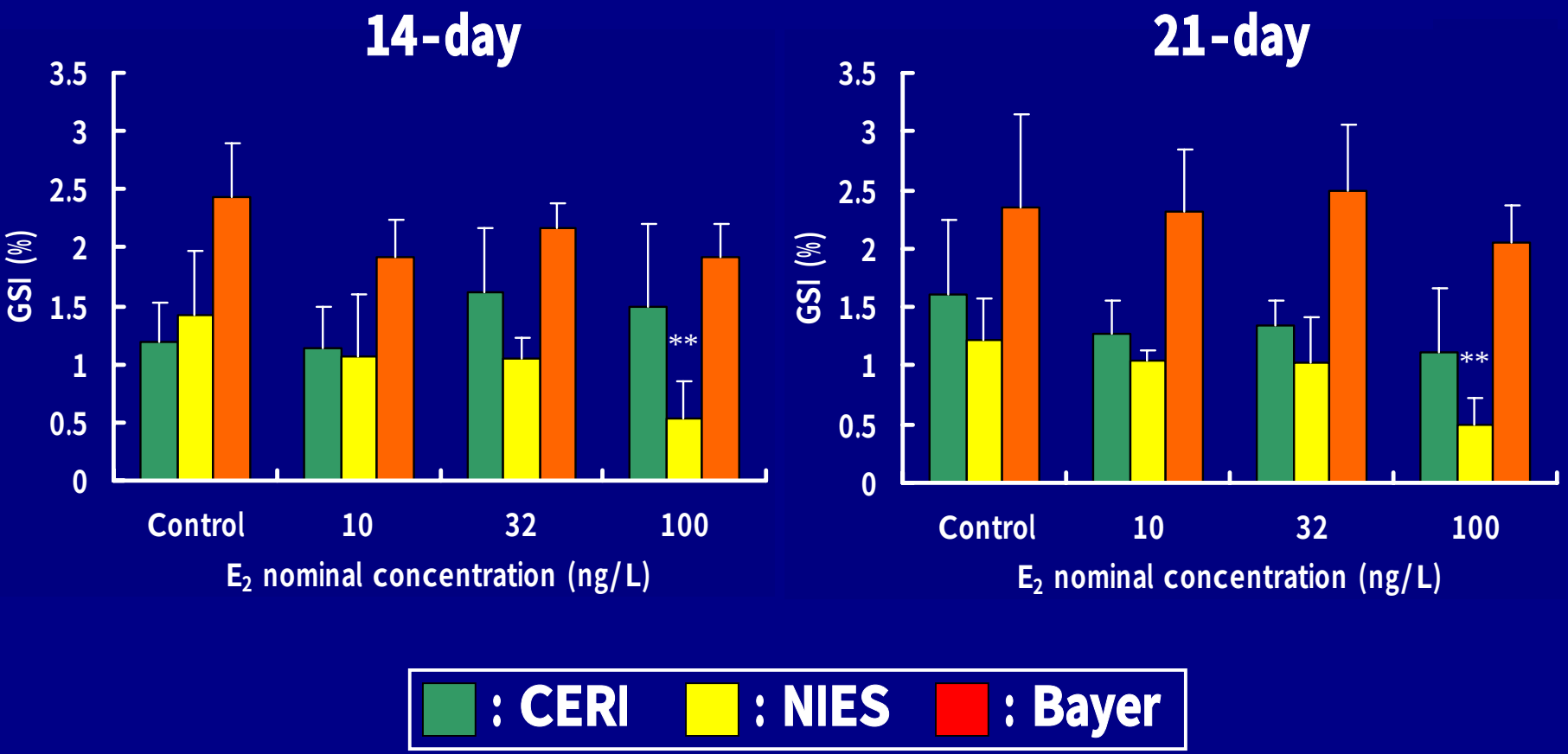
×: Not detected

ND: Not determined, NA: Not available yet

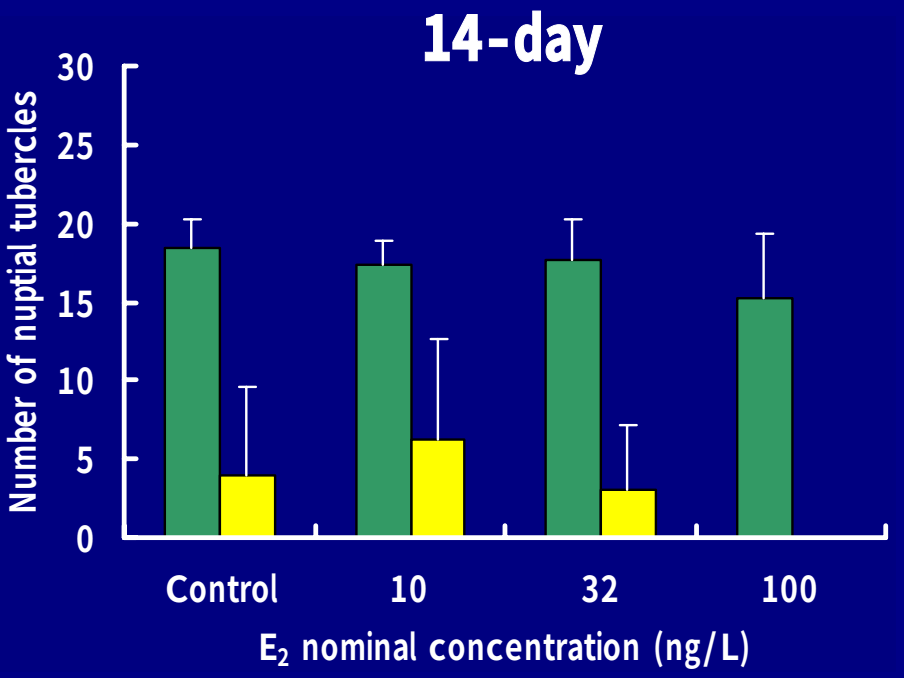
E2 exposure with fathead minnow

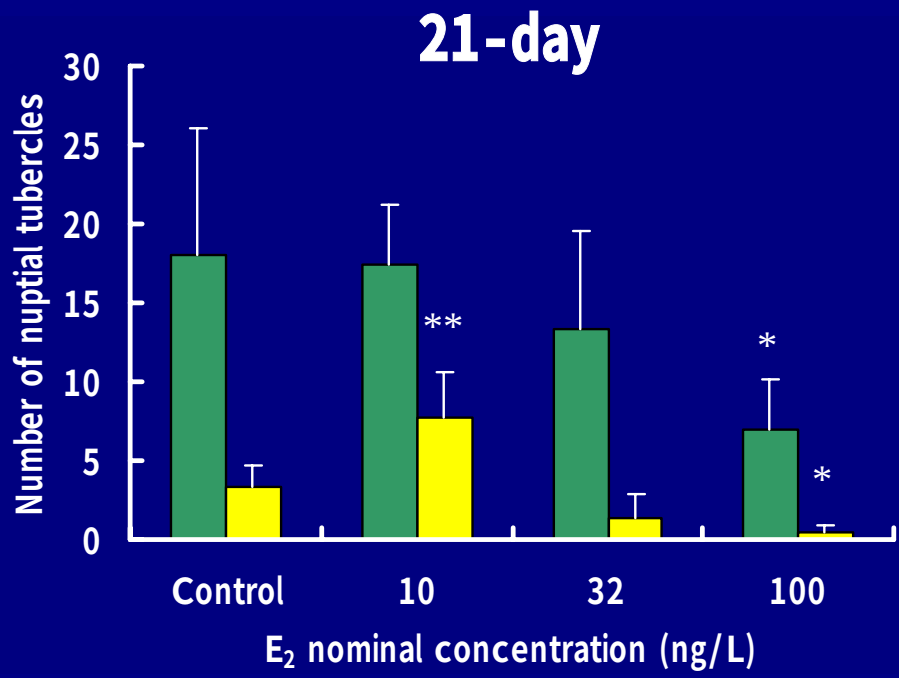
- Laboratory in charge: CERI, NIES, Bayer
- Nominal conc.: 100, 32, 10 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 8 to 9 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 10 or 15 L / vessel
- Dilution water: dechlorinated tap water or reconstituted water
- Test temperature: 25±2℃
- Food: brine shrimp
- Chemical analysis: LC-MC (once a week)
- VTG analysis: ELISA (EnBio or Biosense)

GSI of male fathead minnow



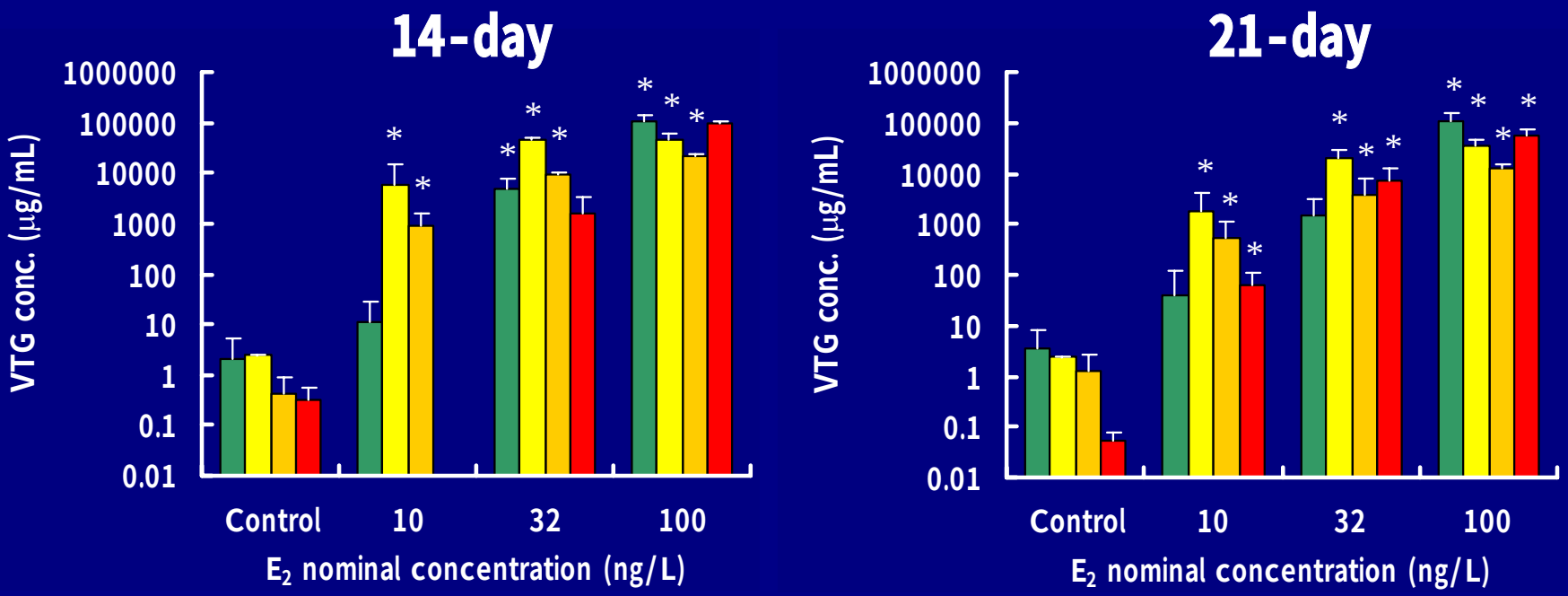
Secondary sexual characters of male fathead minnow





: CERI : NIES

VTG of male fathead minnow



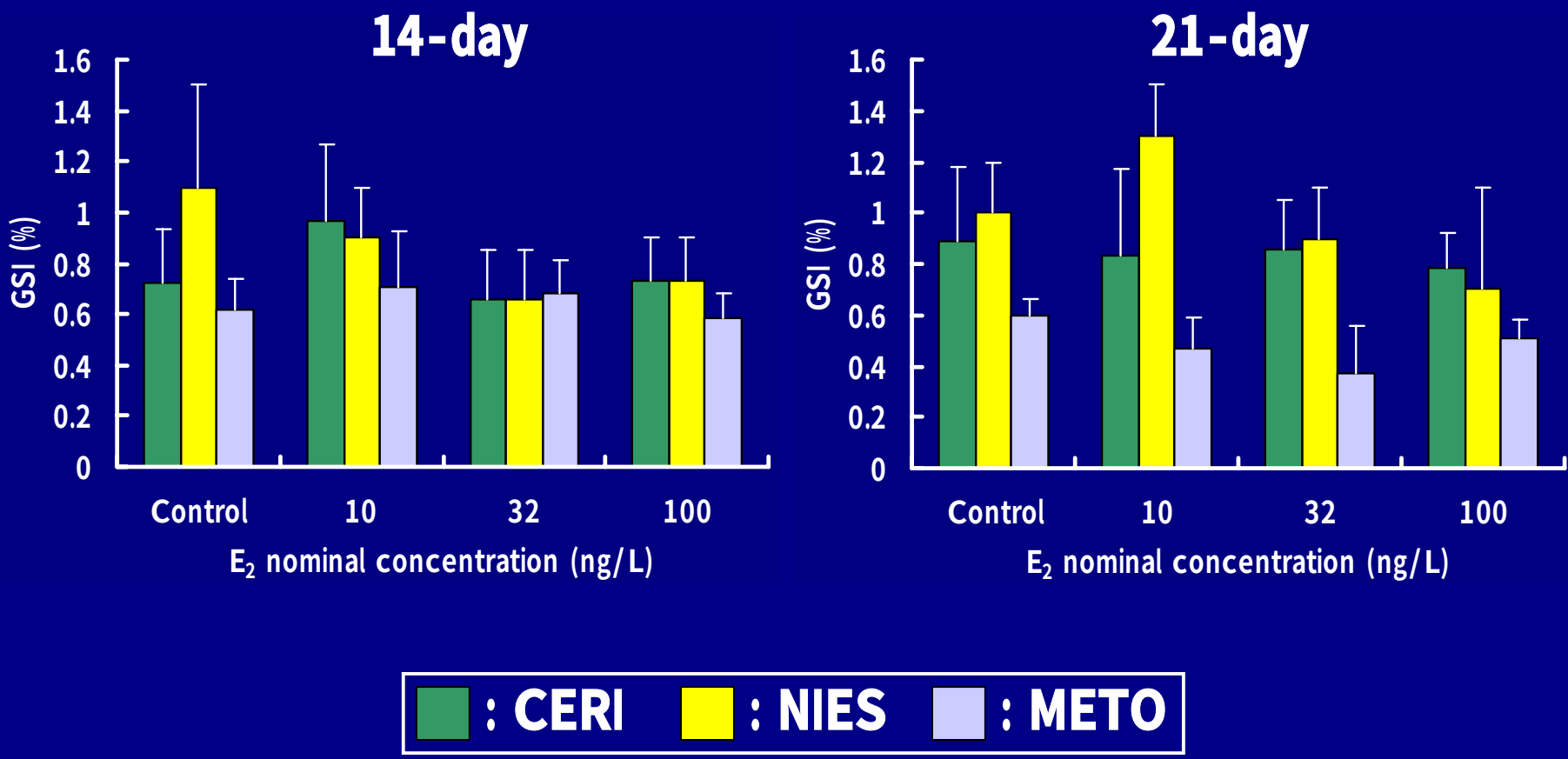
: CERI/EnBio (Blood) : NIES/EnBio (Blood) : NIES/Biosense (Blood) : Bayer/Biosense (Plasma)

The plasma sample from several fish was not obtained due to the technical problem.

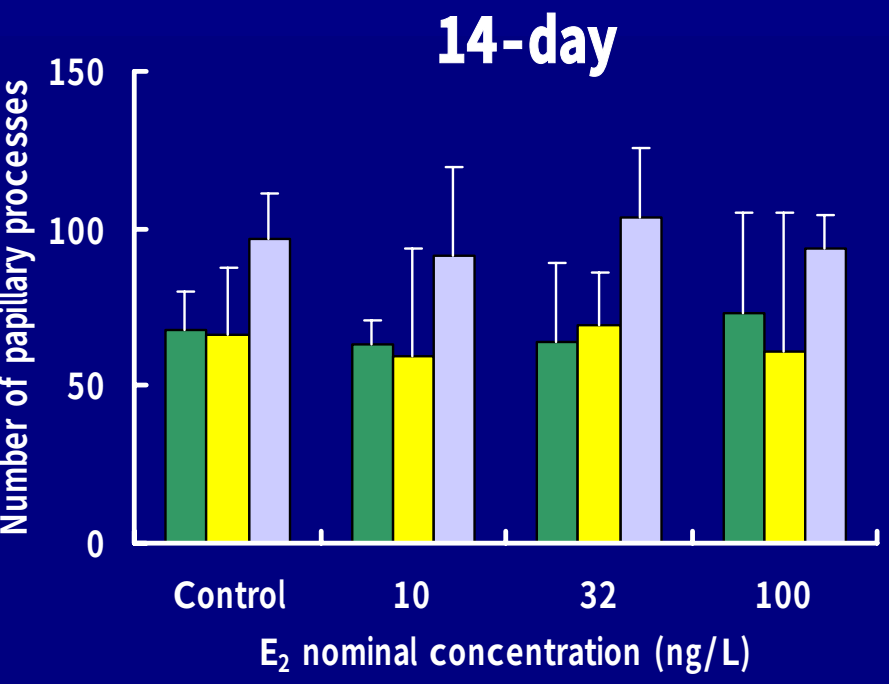
E2 exposure with medaka

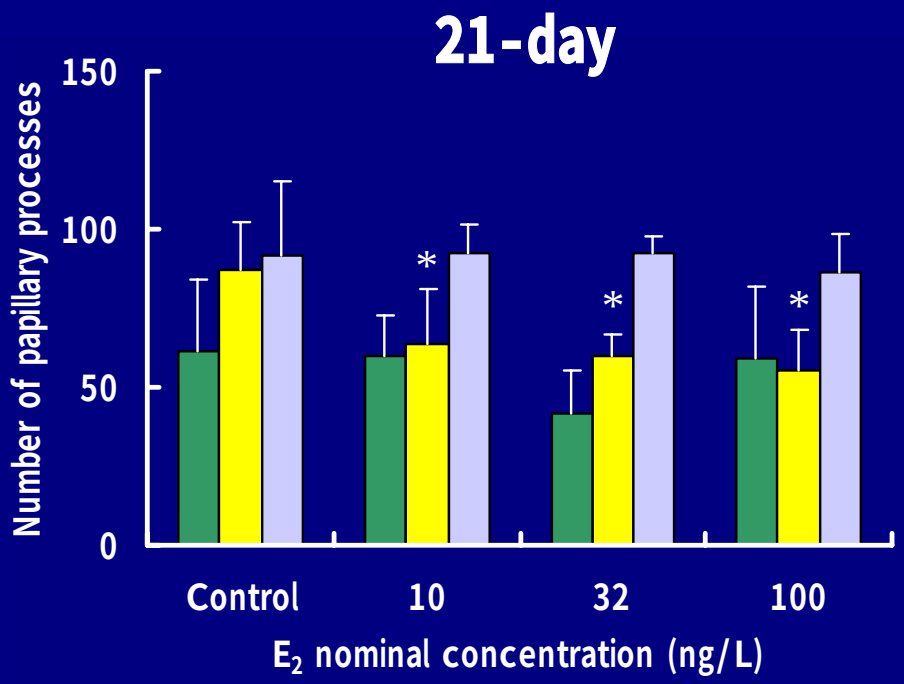
- Laboratory in charge : CERI, NIES, METO
- Nominal conc.: 100, 32, 10 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 4 to 5 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 1.8 to 5 L / vessel
- Dilution water: dechlorinated tap water
- Test temperature: 24±2℃
- Food: brine shrimp (newly hatched larvae)
- Chemical analysis: LC-MC (once a week)
- VTG analysis: ELISA (Transgenic, EnBio or Biosense)

GSI of male medaka



Secondary sexual characters of male medaka



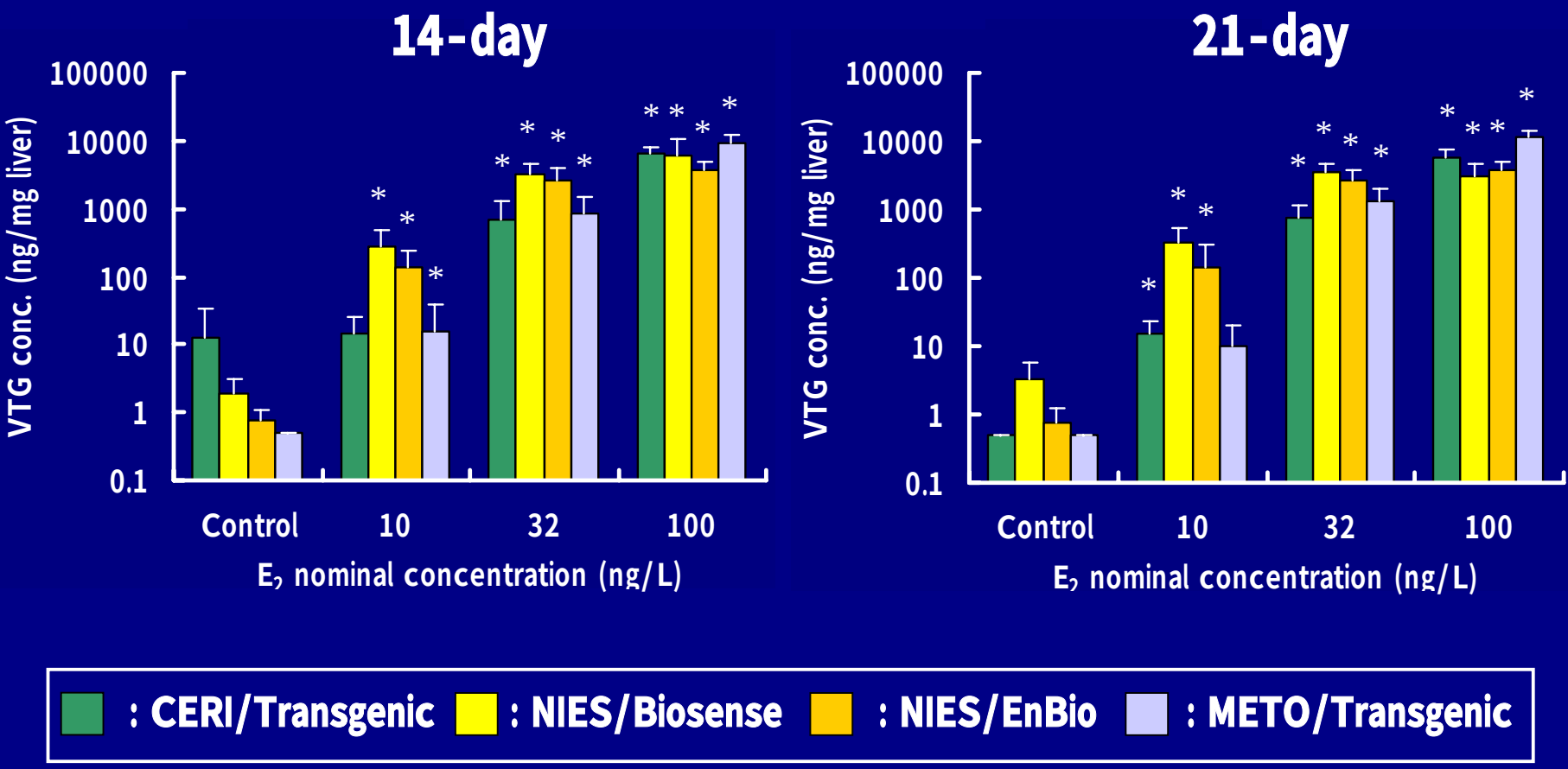


Male

Female

: CERI : NIES : METO

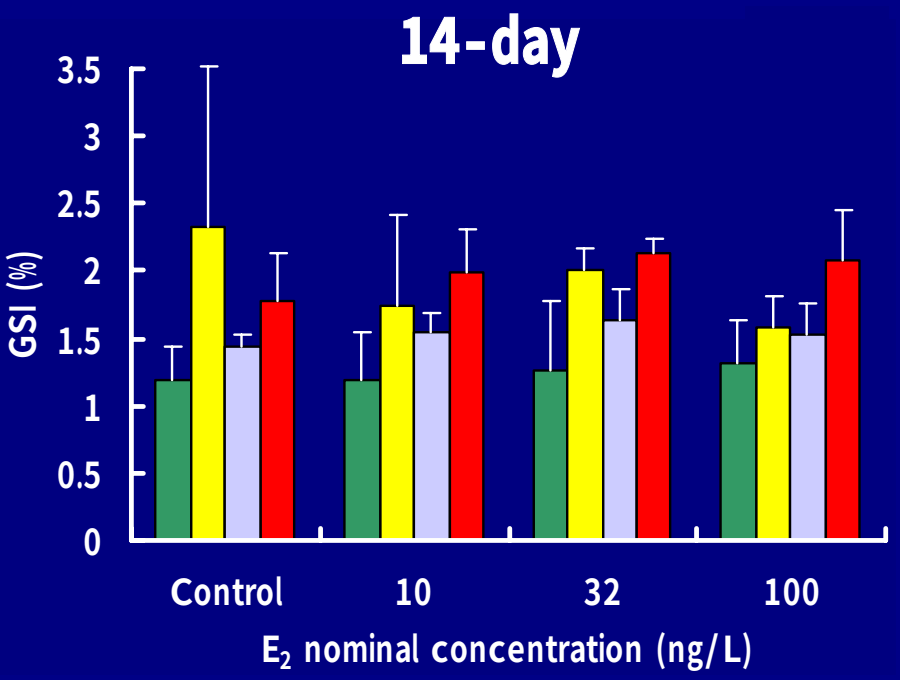
VTG of male medaka

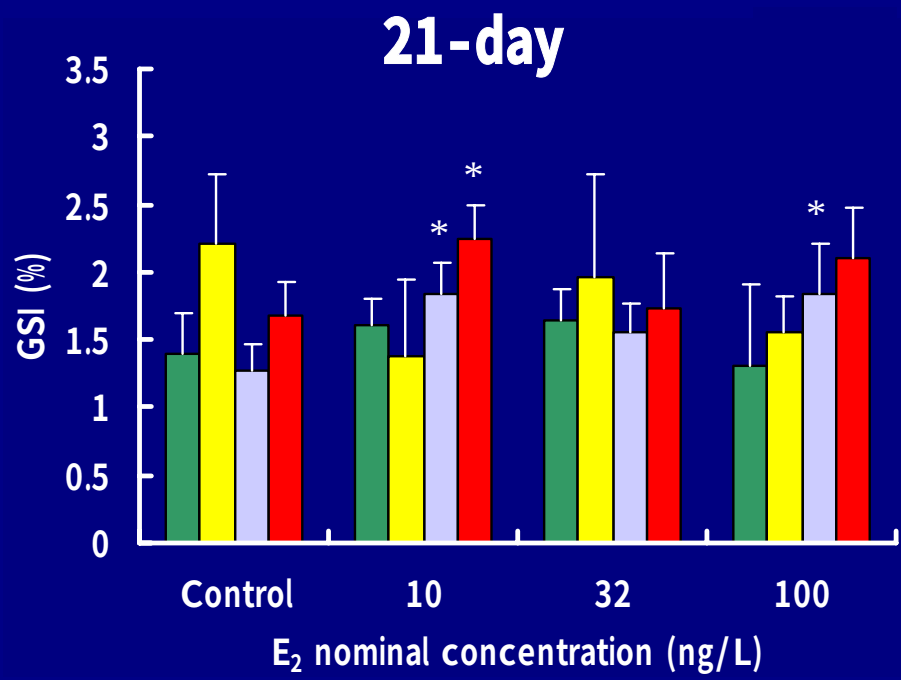


E2 exposure with zebrafish

- Laboratory in charge: CERI, NIES, METO, Bayer
- Nominal conc.: 100, 32, 10 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 4 to 6 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 4.5 to 15 L / vessel
- Dilution water: dechlorinated tap water or reconstituted water
- Test temperature: 26±2℃
- Food: brine shrimp
- Chemical analysis: LC-MC (once a week)
- VTG analysis: ELISA (EnBio or Biosense)

GSI of male zebrafish

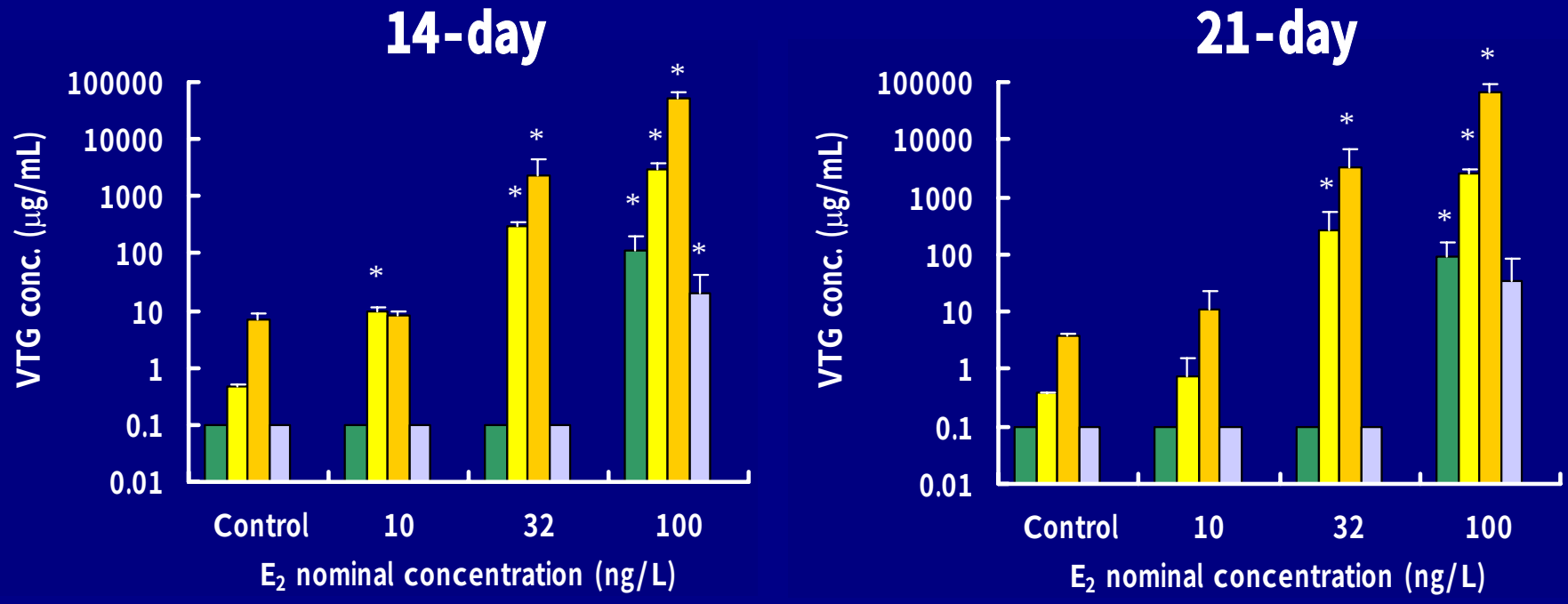




: CERI : NIES : METO : Bayer

One male fish in Bayer was identified as female fish after excision of gonads.

VTG of male zebrafish



: CERI/EnBio (Blood)
: NIES/EnBio (Blood)
: NIES/Biosense (Blood)
: METO/EnBio (Blood)
: Bayer/Biosense (Plasma)

The data from Bayer is not submitted yet.

Draft results of the studies with TB

Test species	Sex	Main endpoint			
		Gross morphology		VTG reduction	Gonad histology
		GSI	2nd sex characters		
Fathead minnow	Male	×	×	×	NA
	Female	△	△	◎	
Medaka	Male	×	×	×	
	Female	△	○	○	
Zebrafish	Male	×	ND	×	
	Female	△		○	

◎: Cleary detected in all studies, ○: Detected in all studies, △: Detected in a few studies,

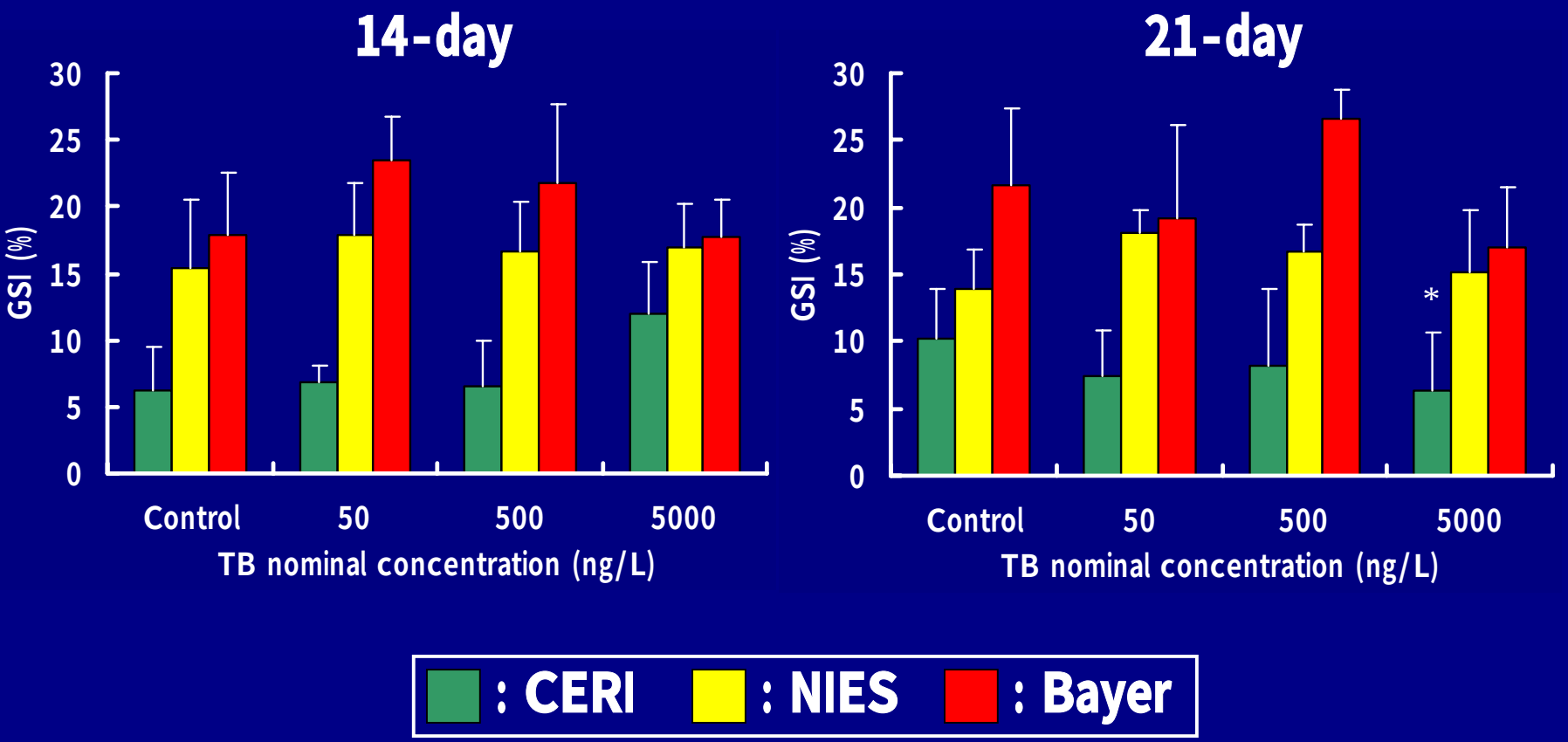
×: Not detected

ND: Not determined, NA: Not available yet

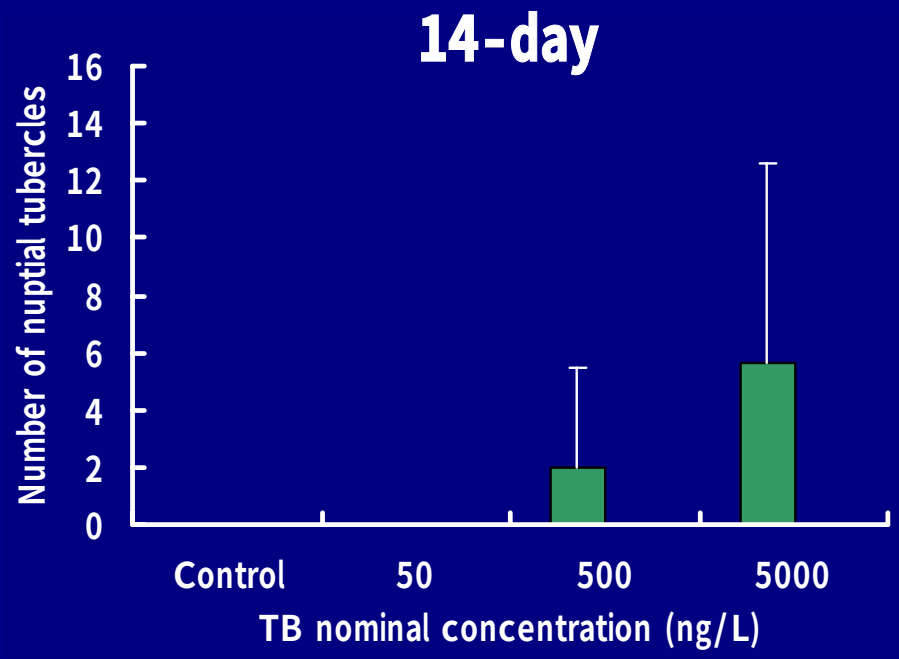
TB exposure with fathead minnow

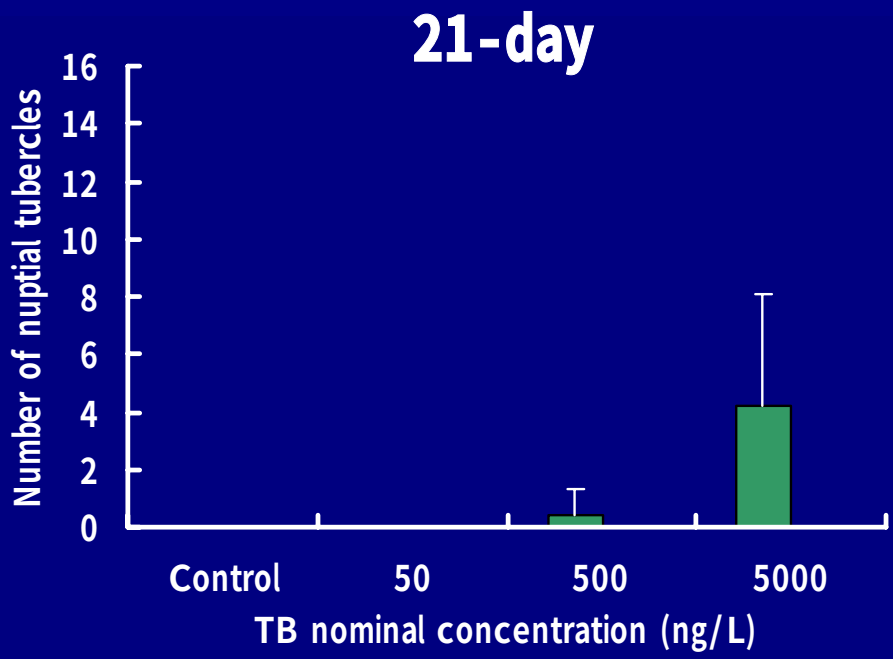
- Laboratory in charge: CERI, NIES, Bayer
- Nominal conc.: 5000, 500, 50 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 8 to 9 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 10 or 15 L / vessel
- Dilution water: dechlorinated tap water or reconstituted water
- Test temperature: 25±2℃
- Food: brine shrimp
- Chemical analysis: GC-MC (once a week)
- VTG analysis: ELISA (EnBio or Biosense)

GSI of female fathead minnow



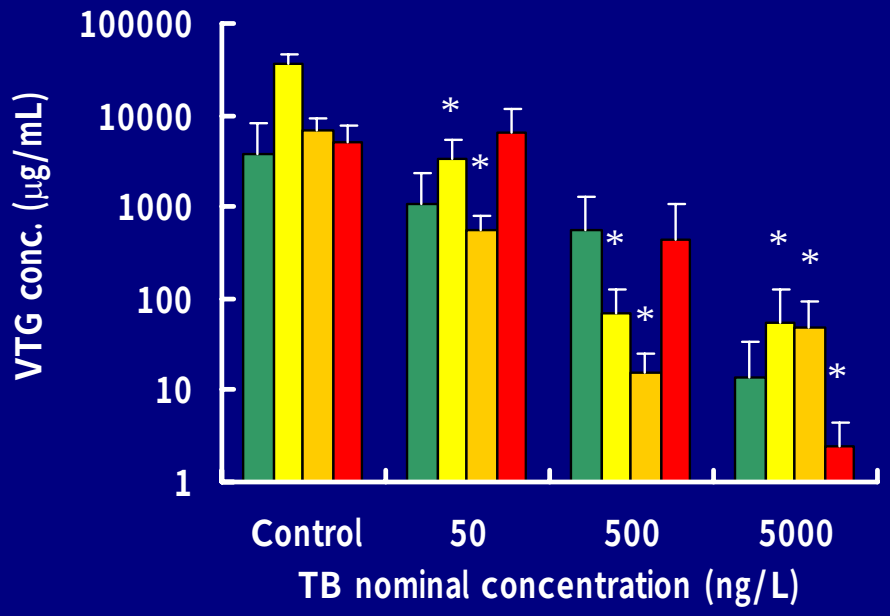
Secondary sexual characters of female fathead minnow

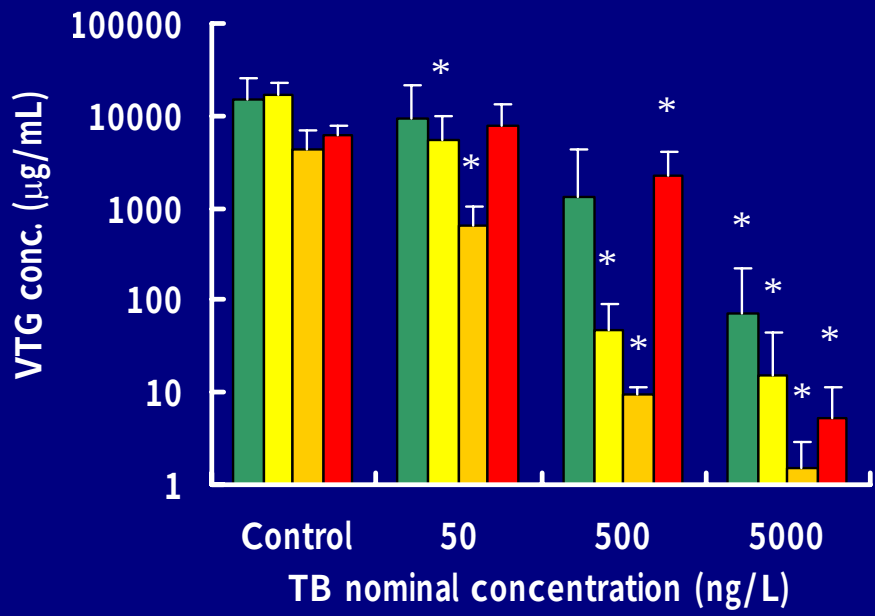




: CERI : NIES

VTG of female fathead minnow





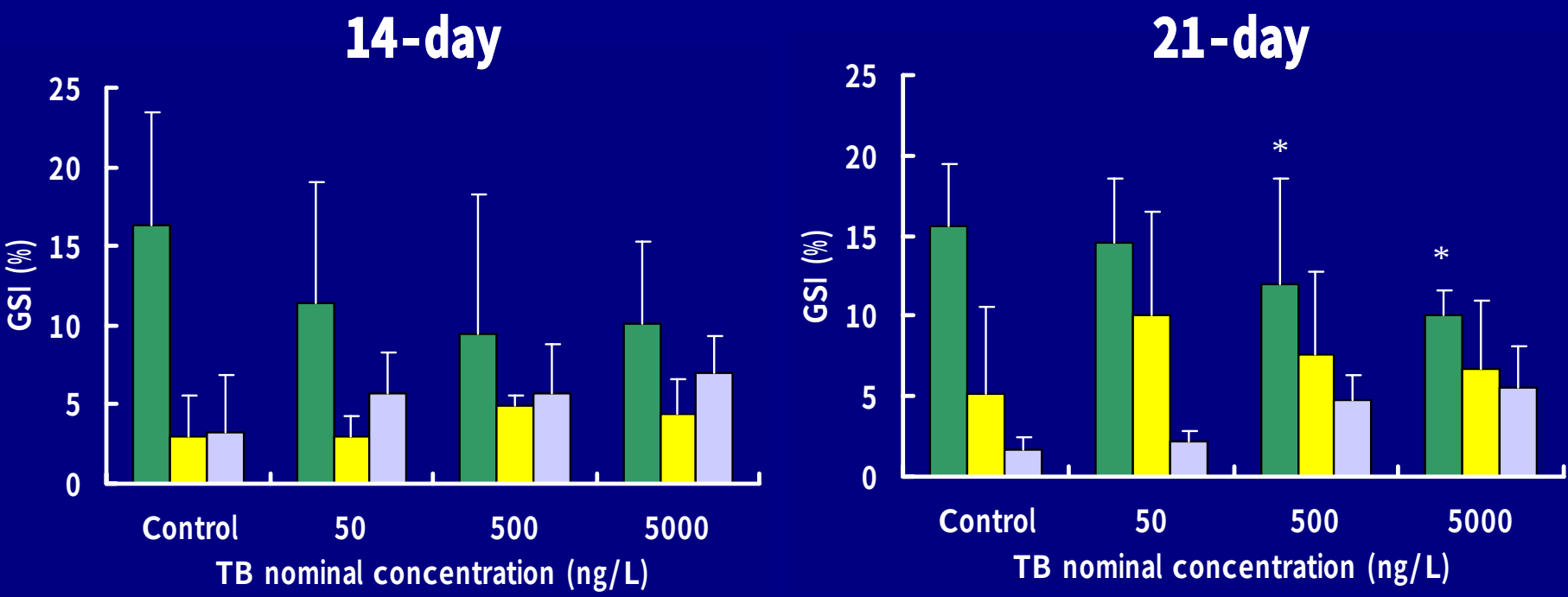
: CERI/EnBio (Blood) : NIES/EnBio (Blood) : NIES/Biosense (Blood) : Bayer/Biosense (Plasma)

The plasma sample from one fish was not obtained due to the technical problem.

TB exposure with medaka

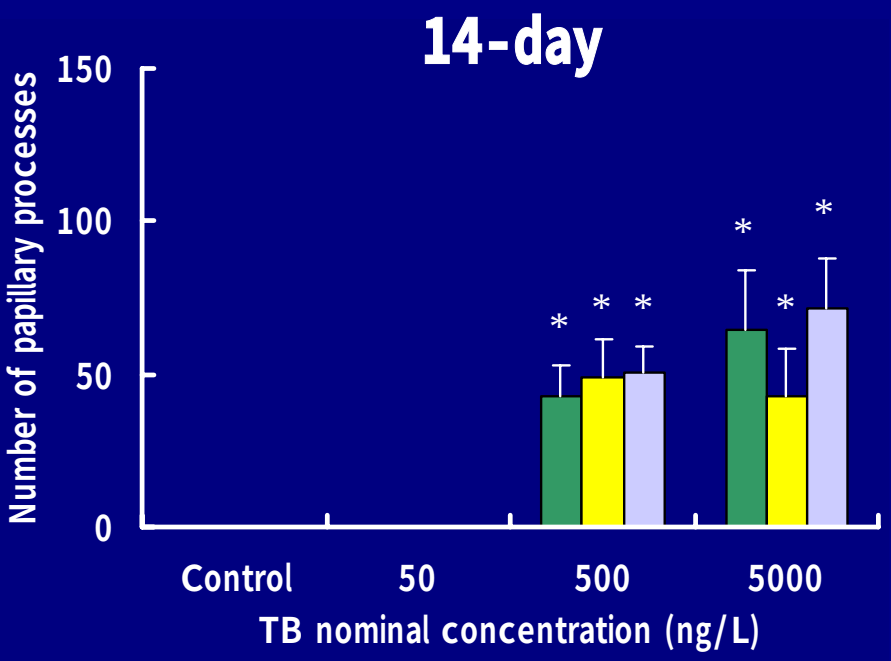
- Laboratory in charge : CERI, NIES, METO
- Nominal conc.: 5000, 500, 50 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 4 to 5 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 1.8 to 5 L / vessel
- Dilution water: dechlorinated tap water
- Test temperature: 24±2℃
- Food: brine shrimp (newly hatched larvae)
- Chemical analysis: GC-MC (once a week)
- VTG analysis: ELISA (Transgenic, EnBio or Biosense)

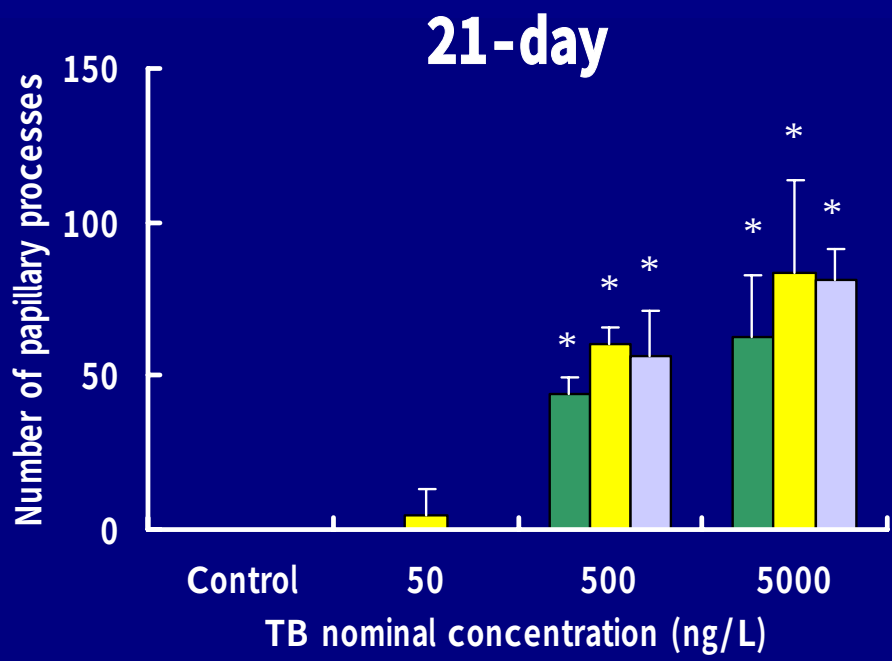
GSI of female medaka



Some females in CERI and METO spawned during the exposure period.

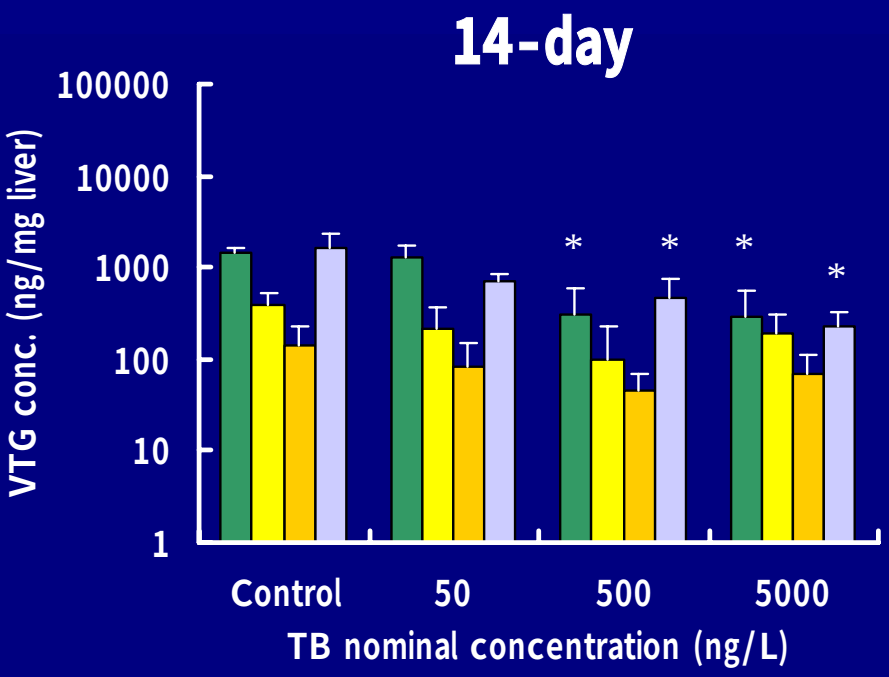
Secondary sexual characters of female medaka

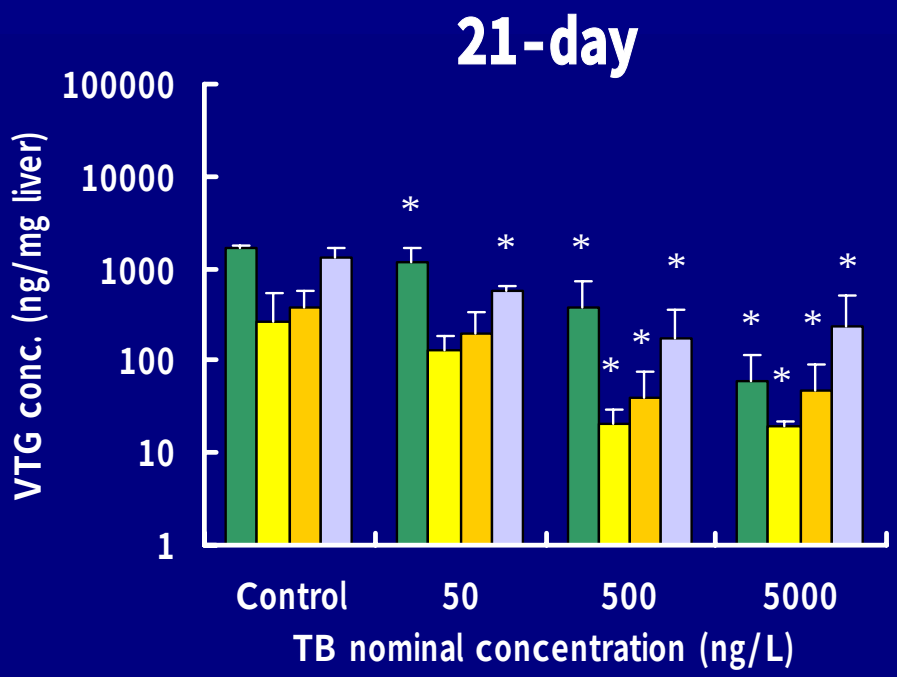




: CERI : NIES : METO

VTG of female medaka



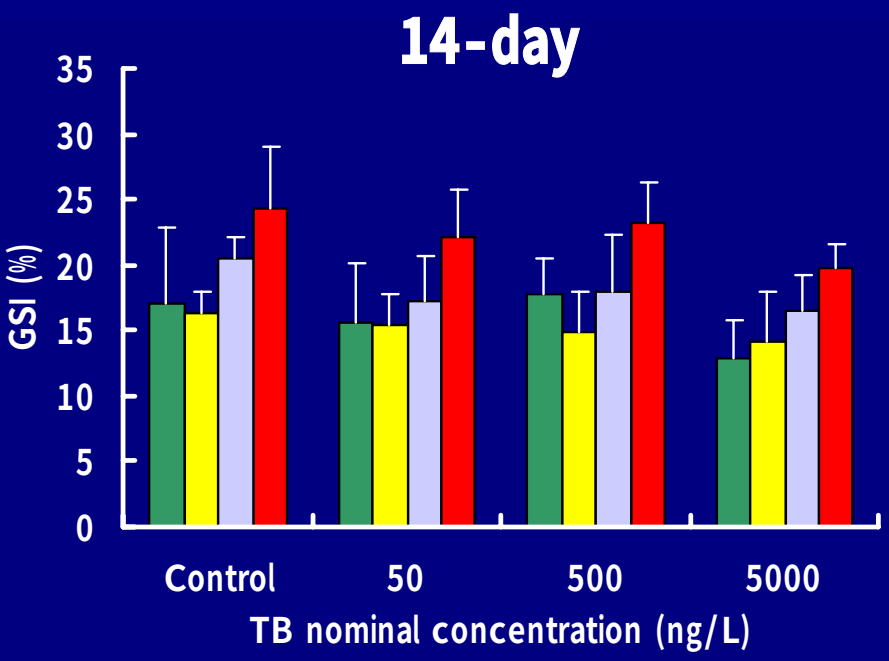


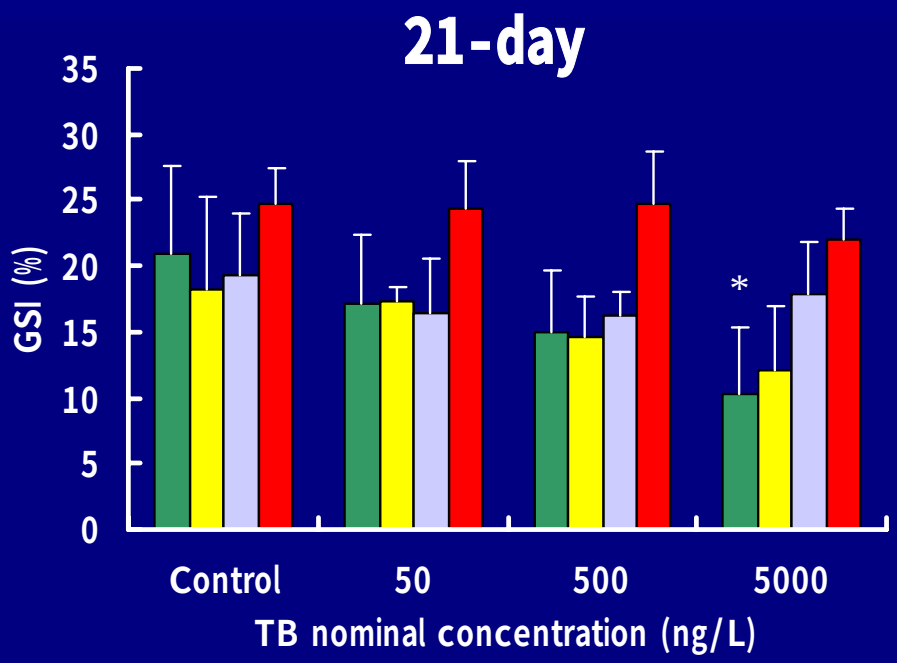
: CERI/Transgenic : NIES/Biosense : NIES/EnBio : METO/Transgenic

TB exposure with zebrafish

- Laboratory in charge: CERI, NIES, METO, Bayer
- Nominal conc.: 5000, 500, 50 ng/L and control
- Number of fish: 10 ♂ and 10 ♀ / treatment
- Age of fish: 4 to 6 month old
- Exposure system: flow-through exposure
- Stock solution: an aqueous solution
- Test solution vol.: 4.5 to 15 L / vessel
- Dilution water: dechlorinated tap water or reconstituted water
- Test temperature: 26±2℃
- Food: brine shrimp
- Chemical analysis: LC-MC (once a week)
- VTG analysis: ELISA (EnBio or Biosense)

GSI of female zebrafish

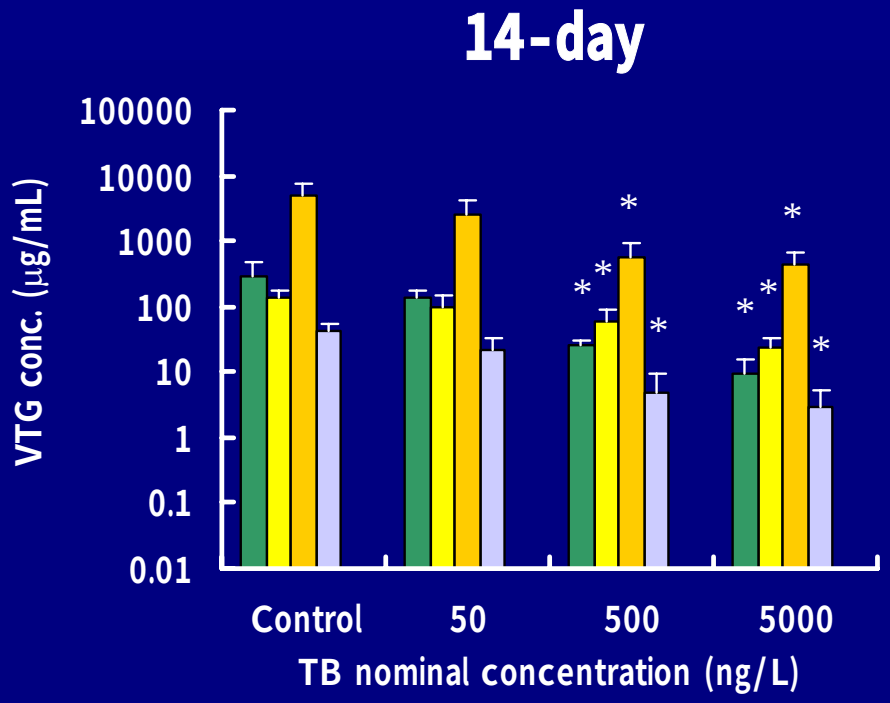


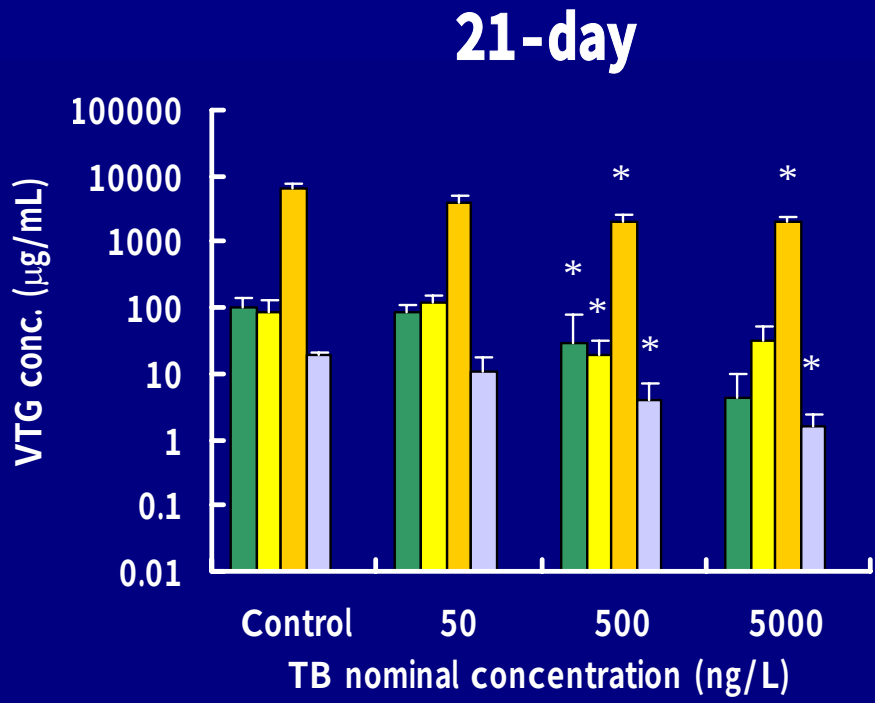


: CERI : NIES : METO : Bayer

Each one male fish in CERI and Bayer was identified as female fish after excision of gonads.

VTG of female zebrafish





: CERI/EnBio (Blood)
: NIES/EnBio (Blood)
: NIES/Biosense (Blood)
: METO/EnBio (Blood)
: Bayer/Biosense (Plasma)

The data from Bayer is not submitted yet.

Draft summary

- ✓ GSI
  - Significant differences were not always observed in all studies.
  - The values in both intra- and inter-laboratory varied considerably possibly due to difference in sexual maturity.
  - Some female medaka and zebrafish spawned during exposure.
- ✓ Secondary sexual characters (fathead minnow and medaka)
  - Significant differences were not always observed in all studies.
  - The quantification method was not standardized between laboratory.
- ✓ VTG
  - Significant differences were observed in all studies.
  - The values in both the control and treatment groups differed with the ELISA kits.
  - The preparation method of the samples was not standardized.

Future plan

- ✓ All data except for gonadal histology will be submitted to the lead laboratory by August 18th.
- ✓ The round-robin evaluation of gonadal histology will be conducted by several fish pathologists in September.
- ✓ The draft report will be submitted to OECD by September 1st and then circulated to the members of the OECD FDG.
- ✓ The final results will be discussed by the OECD FDG meeting which will be held on October 21st and 22nd.

Acknowledgement

- Bayer CropScience AG.
- Chemicals Evaluation and Research Institute, Japan
- METOCEAN ENVIRONMENT INC.
- National Institute for Environmental Studies

- Ms. Anne GOURMELON (OECD)